and also by Cook & Fincham (1969) in this department, that the haemolytic and mitogenic properties of streptolysin S may not be related. This paper reports the separation of these two activities on DEAE-cellulose, thus confirming this suggestion.

It is generally agreed that the haemolytic material is probably implicated in the release of lysosomal enzymes, and it has been suggested (Allison & Malucci, 1964; Hurvitz & Hirschhorn, 1965) that lymphocyte transformation by streptolysin S and certain other mitogens may be initiated directly by the 'unstabilizing' of lysosomal membranes. Experiments with the fractions purified on DEAE-cellulose indicate that release of enzymes from isolated lysosomes occurs only with the haemolytic material (which does not induce lymphocyte transformation). For streptolysin S therefore lymphocyte transformation is not necessarily initiated by lysosomal enzyme release.

McCarty (1948) reported the production of a ribonuclease by certain group A streptococci, and the streptolysin S produced in this laboratory has been found to contain ribonuclease. Even after a tenfold purification has been obtained, the mitogenic material contains most of the original ribonuclease activity. Both the mitogenic material and the ribonuclease are heat-stable, are inactivated by proteinases, and are of similar molecular weight. The possibility that lymphocyte transformation is initiated by the ribonuclease is therefore under consideration. In this connexion it is relevant to note that Cooper & Rubin (1965) showed that degradation of RNA occurs immediately after the addition of phytohaemagglutinin (PHA) to lymphocyte cultures. They consider that 'the primary effect of PHA on lymphocytes may be the degradation of pre-existing RNA, including stable messenger RNA, concerned with the maintenance of the resting state, causing interruption of resting cell processes and permitting the onset of growth'. Such degradation presumably occurs by release of ribonuclease from the lysosomes. If streptococcal ribonuclease proves to induce transformation it may be unnecessary to invoke any lysosome-labilizing effect.

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The Metabolism of 4-Ethylsulphonyl-naphthalene-1-sulphonamide

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The bladder carcinogen, 4-ethylsulphonyl-naphthalene-1-sulphonamide (ENS), induces an acute proliferative response in mice (Clayson, Pringle & Bonser, 1967). Changes in nucleic acid synthesis occur within a few hours of its administration (Lawson, Dzhirov, Lewis & Clayson, 1968; Levi, Cowen & Cooper, 1969). Therefore the distribution and metabolism of [³⁵S]ENS, labelled in the sulphonamide group (Turner & Dean, 1969), have been investigated.

Male or female A×IFF₁ hybrid mice were starved for 4 hr., and then given 0.3 ml. of water followed 4 hr. later by 0.5 mg. (4.5 μg) of [³⁵S]ENS in aqueous propylene glycol by stomach tube. The ³⁵S content of the tissues was estimated by liquid-scintillation counting after hydrogen peroxide-perchloric acid oxidation (Mahin & Lofberg, 1966).

The chemical was rapidly absorbed and 50% of the ³⁵S appeared in the urine in 12 hr. Even 5 hr. after dosing the concentration of ³⁵S in the urine was ten times as great as that in the blood. At 12 hr. the ³⁵S content of the tissues was: stomach <2%; intestines 6–10%; kidney 1%; liver 6–8% of the dose. The blood concentration was 15–20 μg./ml. (calculated as ENS) and this fell to 1 μg./ml. 96 hr. after treatment. No significant differences between male and female mice were observed.

Degradation of ENS to SO₄²⁻ is a minor metabolic pathway, as less than 0.5% of the radioactivity in 48 hr. urine could be co-precipitated with BaSO₄. Chromatography of ether extracts of the urine showed that unchanged ENS was a major metabolite in urine. Over 10% of the dose was excreted unchanged in the first 12 hr. The other principal constituent of the ether extract was more polar and u.v.-fluorescent and gave reactions for sulphonamide. It was identified chromatographically as 4-(2'-hydroxyethyl)sulphonylnaphthalene-1-sulphonamide (HO-ENS) and its urinary output comprised 5–8% of the dose in 12 hr. Further HO-ENS was obtained by incubation of the urine with β-glucuronidase, when one of the more polar spots on chromatograms of the urine disappeared. This is presumed to be HO-ENS glucuronide.

Another polar fluorescent compound that did not react for sulphonamide was recovered by repeated extraction of the urine with butan-1-ol. It resembled

4-ethylsulphonylnaphthalene-1-sulphonic acid in its chromatographic behaviour and u.v. spectrum. Metabolic deamination of a sulphonamide does not appear to have been previously recorded.

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Distribution and Biological Role of 3-Methylhistidine in Actin and Myosin

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3-Methylhistidine is present in actin from adult and foetal skeletal muscle, whereas it is only found in myosin of skeletal muscle shortly after birth in the rabbit (Johnson, Harris & Perry, 1967; Asatoor & Armstrong, 1967; Trayer, Harris & Perry, 1968).

In extension of these findings, actin from red skeletal and cardiac muscles of the rabbit and from smooth muscle of the cow uterus and from certain invertebrate muscles has been found to give 3-methylhistidine:histidine ratios similar to that (1:7.6) of purified actin from adult rabbit white skeletal muscle. Slight differences in some of the ratios may be due to small amounts of impurities in the actin preparations.

Myosins from red and cardiac muscles had 3-methylhistidine:histidine ratios much lower (from 1:79 to 1:178) than that (1:44) of purified myosin from adult rabbit white skeletal muscle. In the cases of myosin from smooth and invertebrate muscles the 3-methylhistidine:histidine ratios were higher than 1:44, probably due to actin contamination.

In the white skeletal muscle from vitamin E-deficient dystrophic rabbits, the 3-methylhistidine:histidine ratio for myosin was significantly lower (values ranging from 1:70 to 1:149), and the ratio for actin was unchanged.

These and earlier suggestions (Trayer, Harris & Perry, 1968) that, at least in the case of myosin, the 3-methylhistidine residues were probably not directly involved in the biological activities of the molecule, were confirmed by studies on the Methylene Blue-sensitized photo-oxidation of puri-

fied rabbit myosin (Stracher & Chan, 1964) and purified rabbit actin (Martonosi & Gouvea, 1961).

After 35 min. of photo-oxidation the adenosine triphosphatase and actin-combining activities of rabbit myosin were not decreased while the content of 3-methylhistidine was reduced to 0.1 mole/mole. Actin polymerization was totally inhibited after 20 min. of photo-oxidation, whereas approximately half of the actin molecules still possessed intact 3-methylhistidine residues. In both proteins, histidine and 3-methylhistidine residues were destroyed at similar rates.

Thus, although 3-methylation of histidine residues in actin and myosin occurs in a wide variety of muscles, it is not essential for the biological activities of these proteins. It is noteworthy that, whereas actin is methylated to a similar extent in all types of muscle, methylation of myosin is greatest in the fastest muscles, less in slower muscles and absent from the foetal type.

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The Immunochemistry of *Shigella flexneri* O-Antigens: a Comparative Analysis of the O-Specific Side-Chain Structures of Serotypes 1a and 2a

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The *Shigella flexneri* lipopolysaccharides are a family of serologically heterogeneous but structurally related polymers, which resemble their *Salmonella* analogues in possessing a common basal structure to which the various O-specific side chains that determine serological behaviour are attached (Simmons, 1966). The molecular configurations of these O-specific side chains, all of which contain D-glucose, N-acetylglucosamine and L-rhamnose bound in sequences that are unique to each serotype, are presently under study to elucidate the stereochemical basis of their serological specificity and cross-reactivity. The structure of serotype 1a lipopolysaccharide has been elucidated by quantitative analysis of neutral oligosaccharides by using borohydride reduction, periodate oxidation, Morgan-Elson and specific enzyme techniques as described